IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Dean Engelhardt et al.

Serial No.: 08/486,069

Filed: June 7, 1995

For: **NUCLEIC ACID SEQUENCING PROCESSES USING MODIFIED NUCLEOTIDES OR NUCLEOTIDE ANALOGS, AND OTHER PROCESSES FOR NUCLEIC ACID DETECTION AND CHROMOSOMAL CHARACTERIZATION USING SUCH MODIFIED NUCLEOTIDES OR NUCLEOTIDE ANALOGS**

Group Art Unit: 1631

Ex'r: Ardin H. Marschel, Ph.D.

South Portland, Maine 04106

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF DR. ALEX A. WALDROP, III

I, Alex A. Waldrop, III, hereby declare as follows:

1. Since 2000, I have been the sole proprietor of my own start-up company having a principal place of business at the Center for Environmental Enterprise (CEE), South Portland, Maine.[1] My present research work focuses on acridine compounds, such as 9-acridinecarbonylimidazole (AcriGlow™ 301), for use in chemiluminescent assays for medical and environmental diagnostics. My professional experience includes research at several organizations, including Maine Medical Center Research Institute, South Portland, Maine (1994 to 2000), IDEXX

[1] CEE is a private, non-profit organization funded by the State of Maine as a business incubator. Located on the campus of Southern Maine Community College, CEE helps new and young firms like my own to commercialize technologies in the environmental field.

Laboratories, Inc., Westbrook, Maine (1992-1993), and Gen-Probe, Inc., San Diego, California (1985-1992) as described in my *curriculum vitae* (cv).[2] Over the past several years I served as a consultant for companies such as Brims Ness, Capricorn Products, Inc., Maine Standards, and Enzo Biochem, Inc.

2. My education and research experience are listed in my cv. I received my bachelor of science degree (B.S.) from the University of Virginia in 1970, graduating with high distinction (*magna cum laude*). In 1977 I received my doctoral degree (Ph.D.) in biophysics from The Johns Hopkins University, Baltimore, Maryland. While at Johns Hopkins, I trained in the Department of Biophysics as a pre-doctoral fellow in the laboratory of Dr. Michael Beer from 1970-1977. I developed multiple heavy atom stains for electron microscopy of nucleic acids. My doctoral dissertation was titled "Chemical Studies of *bis*(Pyridine)osmate(VI) Esters and the Mercury Enhancement of Osmium Labelling of Polynucleotides" [Dissertation Abstracts International 38 (11-B):5354+ (194 pp.) (1978)]. As a postdoctoral fellow, I worked in the laboratory of Dr. David C. Ward at Yale University, New Haven, Connecticut from 1977-1980. While at Yale I used reactions with heavy metal intermediates to synthesize detectable non-radioactively modified nucleotides. I contributed to the discovery that these modified nucleotides could be incorporated *in vitro* into nucleic acids for use as non-radioactive nucleic acid probes. This discovery led directly to the development of several non-radioactively modified nucleotides and nucleotide analogs which are used for *in situ* gene and nucleic acid detection. These modified nucleotides and nucleotide analogs and their use in detection processes are described in several U.S. patents (Nos. 4,711,955; 5,328,824; 5,449,767; and 5,476,928). I am one

[2] **Copy attached as Exhibit 1.**

of three inventors listed on these patents.[3] These modified nucleotides and nucleotide analogs include biotinylated nucleotides and other labeled nucleic acid compositions which have been sold commercially for years.

3. After my postdoctoral work, I was Assistant Professor of Chemistry at the University of Virginia, Charlottesville, Virginia, from 1980-1982. While working in the UVA Department of Chemistry, I taught undergraduate biophysical chemistry. I also prepared nucleotide derivatives of tubercidin and characterized allylamine derivatives. From 1982-1985, I was Research Associate in the Department of Microbiology at UVA where I worked on several projects including the development of a new DNA sequencing method and a gel filtration method for nucleotide purification and desalting, and the synthesis of a series of 5'-thymidine triphosphate derivatives and a dUTP analog containing an ethylenediamine-tetraacetic acid (EDTA) group.

4. I am the author of five scientific publications and I am also an inventor on seven U.S. patents, including the four patents referenced in paragraph 2 above.

5. Enzo Life Sciences, Inc. has requested that I review as its scientific consultant significant portions of the most recent prosecution history of United States Patent Application Serial No. 08/486,069, filed on June 7, 1995 ("the '069 application") in the name of Dean L. Engelhardt, et al. as inventors.[4] The title of

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[3] All four of these U.S. patents name David C. Ward, Pennina R. Langer and Alexander A. Waldrop, III, as co-inventors. U.S. Patent No. 4,711,955 is titled "Modified Nucleotides and Methods of Preparing and Using Same" and it issued on December 8, 1987. U.S. Patent No. 5,328,824 is titled "Methods of Using Labeled Nucleotides" and it issued on July 12, 1994. U.S. Patent No. 5,449,767 is titled "Modified Polynucleotides and Methods of Preparing Same," having issued on September 12, 1995. The fourth, U.S. Patent No. 5,476,928, is titled "Modified Nucleotides and Polynucleotides and Complexes Formed Therefrom," and it issued on December 19, 1995.

[4] I am also the same Alex Waldrop who submitted a Supplemental Declaration earlier this month in U.S. Patent Application Serial No. 08/479,997, which is related to the '069 application. I understand that both applications share the same specification which I have read in both instances.

the Engelhardt application is "Nucleic Acid Sequencing Processes Using Modified Nucleotides or Nucleotide Analogs, And Other Processes for Nucleic Acid Detection And Chromosomal Characterization Using Such Modified Nucleotides or Nucleotide Analogs." Included for this particular review were the following documents:

- two Office Actions dated July 1, 2003 and March 12, 2003;
- five documents cited in the July 1, 2003 Office Action:
    - Kourilsky et al., U.S. Patent No. 4,581,333 and Great Britain Patent Application Publication No. 2,019,408 A, published October 31, 1979;
    - Langer et al., "Enzymatic synthesis of biotin-labeled polynucleotides: Novel nucleic acid affinity probes," Proc. Natl. Acad. Sci. (USA) 78:6633-6637 (November 1981);
    - Dale et al., "Mercurated Polynucleotides: New Probes for Hybridization and Selective Polymer Fractionation," Biochemistry 14:2458-2469 (1975);
    - Dunn et al., "A Novel Method to Map Transcripts: Evidence for Homology between an Adenovirus mRNA and Discrete Multiple Regions of the Viral Genome," Cell 12:23-36 (1977); and
    - Hartman et al., "Methacrylate Polymerization by AzoRNA: Potential Usefulness for Chromosomal Localization of Genes," Biopolymers 20:2635-2648 (1981);
- Several of Applicants' responses that were filed in the '069 application:
    - December 31, 2003 Amendment;
    - July 13, 2004 Supplemental Amendment;
    - September 14, 2004 Second Supplemental Amendment; and

- a draft of their September 28, 2004 Third Supplemental Amendment;[5,6]
- the patent specification filed on June 7, 1995 (but claiming priority to June 23, 1982) [hereinafter "the '069 specification"]; and
- three legal cases:
  - *In re Grasselli* (PTO Board of Appeals & Interferences, 1983);
  - *Ex parte Pearson* (PTO Board of Appeals & Interferences, 1985); and
  - *Ex parte Parks* (PTO Board of Appeals & Interferences, 1993).

I am being compensated for my review and for making this Declaration.[7]

6. Based upon my review of the claims being submitted to the U.S. Patent Office as part of the Third Supplemental Amendment, I understand that the invention in the '069 application is directed to processes for nucleic acid sequencing, detection and chromosomal characterization. These processes utilize detectable nucleic acids comprising modified or labeled nucleotides or modified or labeled nucleotide analogs. In certain of the processes claimed in the '069

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[5] I have been informed that my Declaration will be submitted to the U.S. Patent Office as part of this Third Supplemental Amendment.

[6] A complete listing of the claims is being provided in the Third Supplemental Amendment. I was provided with a copy of the following claims which are pending in the '069 application: 569-571, 573-575, 577, 582-589, 592-594, 597-600, 602-604, 607-608, 610-612, 614-624, 634-635, 637-638, 641-642, 646, 648-651, 656-661, 667, 670, 707-714, 716-717, 719-723, 725-727, 729, 734-747, 749-752, 754-756, 759-760, 762-764, 766-776, 786-787, 789-790, 793-794, 796-797, 800-803, 808-813, 819, 822, 859-866, 868-869, 871-875, 877-879, 881, 886-899, 901-904, 906-908, 911-912, 914-916, 918-928, 938-939, 941-942, 945-949, 952-955, 960-965, 971, 974, 1011-1018, 1020-1021, 1023-1027, 1029-1031, 1033, 1038-1051, 1053-1056, 1058-1060, 1063-1064, 1066-1068, 1070-1080, 1090-1091, 1093-1094, 1097-1099, 1101, 1104-1107, 1112-1117, 1123, 1126, 1163-1170, 1172-1173, 1175-1179, 1181-1183, 1185, 1190-1200, 1204, 1208-1209, 1212-1216, 1218-1244, 1248-1249, 1253, 1255-1258, 1263-1270, 1272, 1275, 1278-1294, 1296-1328, 1331-1332, 1334-1351, 1353-1354, 1357-1358, 1360, 1362-1369, 1372-1380, 1383, 1386-1391, 1393-1407, 1409-1487, 1490-1491, 1493-1516, 1518, 1520-1525, 1527, 1530-1539, 1541, 1544-1568, 1570-1585, 1587, 1592-1612, 1614-1615, 1618-1621, 1623-1628, 1631-1632, 1635-1647, 1649-1656, 1658, 1660-1667, 1670-1677, 1679-1680, 1682, 1685-1773 and 1775-1796.

[7] I was also compensated for making the Supplemental Declaration submitted in Serial No. 08/479,997 and referenced in footnote 4 above.

application, such modified or labeled nucleotides or modified or labeled nucleotide analogs comprise an element that is termed "Sig," and which is further defined as a detectable non-radioactive *non-nucleotidyl* moiety that comprises at least three carbon atoms. Two process claims in which Sig is a detectable non-radioactive *non-nucleotidyl* moiety are claims 1298 and 1582.[8,9] Both claims are independent. The former claim is a process for detecting a nucleic acid of interest in a sample; the latter is a process for preparing a detectable non-radioactively labeled oligo- or polynucleotide of interest.

7. I have been informed by Ronald C. Fedus, Esq. that my Supplemental Declaration submitted in Serial No. 08/479,997 (and referenced in footnote 4 above), was discussed at an interview held on September 21, 2004 among the Patent Examiner, a representative for the Applicants, and Mr. Fedus. According to Mr. Fedus, the Patent Examiner was asked whether a declaration directed to the inclusion of the term "non-nucleotidyl" for the Sig moiety should be submitted in the '069 application to support claims 1298 and 1582. The Patent Examiner indicated that the declaration should be submitted with a proper caption to complete the record.[10]

8. As Enzo's scientific consultant, I am making this Declaration in support of the subject matter claimed in the '069 application, and in particular, to the

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[8] Text of claims 1298 and 1582 attached as Exhibit 2.
[9] Claims that depend from claim 1298 include 1299-1328, 1331-1351, 1353, 1354, 1357, 1368, 1360, 1362-1369, 1372-1380, 1383, 1386-1391, 1393-1407, 1409, 1410, 1725, 1726, and 1757-1765). Claims that depend from claim 1582 include 1583-1585, 1587, 1592-1612, 1614, 1615, 1618-1621, 1623-1628, 1631, 1632, 1635-1647, 1649-1656, 1658, 1660-1667, 1670-1677, 1679, 1680, 1682, 1685-1699 and 1760.
[10] I note that no issue involving the term "non-nucleotidyl" was raised in either the July 1, 2003 Office Action or the March 12, 2003 Office Action.

definition recited in claims 1298 and 1582 that the non-radioactive Sig component is "non-nucleotidyl."[11]

9. As set forth in my cv, I am a chemist with substantial experience and background in nucleic acid chemistry. My knowledge, background, training and experience in nucleic acid chemistry encompasses nucleic acid modifications, including labeling nucleic acids for use in hybridization and detection assays. I am familiar with several nucleic acid detection formats and with nucleic acid probe technology in general. My professional and academic career involves extensive research exploring the modifications and labeling of nucleic acids for use as probes in hybridization and detection assays. One of my more recent areas of research is assays using novel chemiluminescent reagents to detect a wide variety of substances including nucleic acids and other biomolecules.

10. Based upon my education, training, background and experience, I believe that at the time the first parent application of the pending '069 application was filed in June 1982, the relevant art to the claimed processes would have included many if not most of the following areas: modifications of nucleic acids, nucleic acid synthesis and labeling, and nucleic acid hybridization, formatting and detection. I consider myself to possess the level of skill, knowledge, training and experience of at least a person skilled in the art to which the present '069 invention pertains.

11. I understand that a patent specification describes the subject matter of a claim if the specification conveys with reasonable clarity to a person skilled in the art, that the inventors were in possession of the subject matter recited in that claim. I also understand that to satisfy the written description requirement, the

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[11] Because the issue of the term "non-nucleotidyl" concerns the same specification for both applications, my statements in this Declaration will largely follow the statements made in my Supplemental Declaration filed in Serial No. 08/479,997.

inventors do not have to utilize any particular form of disclosure to describe the subject matter of the claim under consideration. For instance, support for the claimed invention may be found in the working examples, in the general description of the invention, or in a combination of the examples and the general description.

12. As a person skilled in the art, it is my opinion and conclusion that the '069 specification reasonably conveys that at the time their application was filed in June 1982, Applicants were in possession of the claimed invention for processes directed to a non-nucleotidyl Sig component. For reasons which are given below, I believe that the '069 specification reasonably conveys that the Sig component is non-nucleotidyl. Furthermore, as explained below, I believe that any inference from the '069 specification and pending claims that the Sig component is or could be nucleotidyl in nature, or that Sig comprises a nucleotide, would be erroneous and unreasonable.

NONE OF THE EXAMPLES FOR THE SIG LABEL MOIETY ARE NUCLEOTIDYL OR A NUCLEOTIDE

13. At the outset I find it significant that the '069 specification discloses several examples for the Sig component -- all of which show that Sig is neither a nucleotide nor that it is nucleotidyl in its nature. Beginning on page 96, last paragraph, and continuing through the first paragraph on page 97, the '069 specification discloses:

> The Sig moiety employed in the make-up of the special nucleotides of this invention could comprise an ***enzyme or enzymic material***, such as ***alkaline phosphatase***, ***glucose oxidase***, ***horseradish peroxidase*** or ***ribonuclease***. The Sig moiety could also contain a ***fluorescing component***, such as ***fluorescein*** or ***rhodamine*** or ***dansyl***. If desired, the Sig moiety could include a ***magnetic component*** associated or attached thereto, such as a ***magnetic oxide*** or ***magnetic iron oxide***, which would make the nucleotide or polynucleotide containing such a magnetic-containing Sig moiety detectable by magnetic means. The

> Sig moiety might also include an ***electron dense component***, such as ***ferritin***, so as to be available by observation. The Sig moiety could also include a ***radioactive isotope component***, such as ***radioactive cobalt***, making the resulting nucleotide observable by radiation detecting means. The Sig moiety could also include a ***hapten component*** or per se be capable of complexing with an antibody specific thereto. Most usefully, the Sig moiety is a ***polysaccharide or oligosaccharide or monosaccharide***, which is capable of complexing with or being attached to a sugar or polysaccharide binding protein, such as a lectin, e.g. Concanavilin A. The Sig component or moiety of the special nucleotides in accordance with this invention could also include a ***chemiluminescent component***. [emphasis added]

Of the approximately twenty different examples for Sig that have been bolded in the passage above, none comprises a nucleotide or the three nucleotidyl subunits (sugar, phosphate and base) necessary to build the nucleotide structure.[12] In contrast, the '069 specification indicates to me that an additional component is required for signal generation, and the three nucleotidyl subunits in and of themselves are not sufficient for generating a signal.

THE SIG LABEL MOIETY IS ATTACHED TO THE BASE, SUGAR OR PHOSPHATE MOIETY OF A NUCLEOTIDE

14. I also find it significant that the '069 specification and the pending claims describe the claimed Sig component in terms of its attachment to the base, sugar or phosphate moiety of a nucleotide.

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[12] **Lehninger was cited in the November 23. 2003 Office Action issued in related Serial No. 08/479,997 and he provides the following description for a nucleotide on page 55, last paragraph:**

> **The recurring structural units of all nucleic acids are eight different *nucleotides*; four kinds of nucleotides are the building blocks of DNA, and four others are the structural units of RNA. Each nucleotide in turn contains three smaller units: [1] a nitrogenous organic base, [2] a 5-carbon sugar, and [3] phosphoric acid . . .**

i) Page 93, first paragraph:

The ***special nucleotides*** in accordance with this invention, as indicated hereinabove, in addition to the P, S and B moieties, include a chemical moiety Sig covalently attached to the P, S and/or B moieties. Of special interest in accordance with the practices of this invention would be those nucleotides having the general formula,

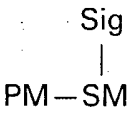
PM—SM—BASE—Sig

wherein P is the phosphoric acid moiety including mono-, di-, tri-, or tetraphosphate, S the sugar or monosaccharide moiety, B the base moiety, either a purine or a pyrimidine. The phosphoric acid moiety P is attached at the 3' and/or the 5' position of the S moiety when the nucleotide is a deoxyribonucleotide and at the 2', 3' and/or 5' position when the nucleotide is a ribonucleotide. The base B moiety is attached from the N1 position or the N9 position to the 1' position of the S moiety when the base moiety is a pyrimidine or a purine, respectively. The Sig moiety is covalently attached to the B moiety of the nucleotide and when so attached is capable of signalling itself or makes itself self-detecting or its presence known and desirably or preferably permits the incorporation of ***the resulting nucleotide*** PM—SM—BASE—Sig into or to form a double-stranded helical DNA or RNA or DNA-RNA hybrid and/or to be detectable thereon.

[emphasis added]

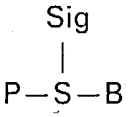
ii) Page 93, second paragraph, through Page 94, second paragraph:

Another special nucleotide in accordance with this invention is characterized by the general formula:



Such nucleotides in accordance with this invention would be characterized as ribonucleotide. The phosphoric acid moiety is attached at the 2', 3' and/or 5' position of the sugar S moiety and the base B being attached from the N1 position or the N9 position to the 1' position of the sugar S moiety when said base is a pyrimidine or a purine, respectively. The Sig chemical moiety is covalently attached to the sugar S moiety and said Sig chemical moiety when attached to said S moiety is capable of signalling itself or making itself self-detecting or its presence known and preferably permits the incorporation of the ***ribonucleotide*** into its corresponding double-stranded RNA or a DNA-RNA hybrid. [emphasis added]

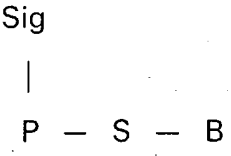
Such nucleotides



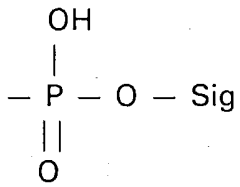
desirably have the Sig chemical moiety attached to the C2' position of the S moiety or the C3' position of the S moiety.

iii) Page 94, last paragraph:

Still further, ***nucleotides*** in accordance with the practices of this invention include the nucleotides having the formula,



wherein P is the phosphoric acid moiety, S the sugar moiety and B the base moiety. In these special nucleotides the P moiety is attached to the 3' and/or the 5' position of the S moiety when the nucleotide is deoxyribonucleotide and at the 2', 3' and/or the 5' position when the nucleotide is a ribonucleotide. The base B is either a purine or a pyrimidine and the B moiety is attached from the N1 or the N9 position to the 1' position of the sugar moiety when said B moiety is a pyrimidine or a purine, respectively. ***The Sig chemical moiety is covalently attached to the phosphoric acid P moiety*** via the chemical linkage



said Sig, when attached to said P moiety being capable of signalling itself or making itself self-detecting or its presence known and desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or DNA-RNA hybrid and when so incorporated therein is still self-detecting. [emphasis added]

iv) Page 97, first full paragraph:

As indicated ***in accordance with the practices of this invention***, the ***Sig component*** could comprise any chemical moiety which is ***attachable*** either directly or through a chemical linkage or linker arm ***to the nucleotide***, such as ***to the base B component therein, or the sugar S component therein, or the phosphoric acid P component*** thereof.

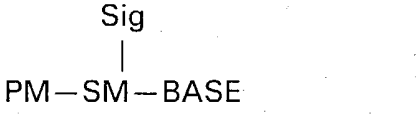
[emphasis added]

v) Originally filed claim 1:

1. ***A nucleotide*** having the general formula PM—SM—BASE—Sig wherein P is the phosphoric acid moiety, S the sugar or monosaccharide moiety, B being the base moiety, the phosphoric acid moiety being attached at the 3' and/or the 5' position of the sugar moiety when said nucleotide is a ribonucleotide, said base being a purine or a pyrimidine, said base being attached from the N1 position or the N9 position to the 1' position of the sugar moiety when said base is a pyrimidine or a purine, respectively, and wherein said Sig is a chemical moiety covalently attached to the base B ***of said nucleotide***, said Sig when attached to said base B being capable of signalling itself or makes itself self-detecting or its presence known. [emphasis added]

vi) Originally filed claim 101:

101. ***A ribonucleotide*** having the general formula,

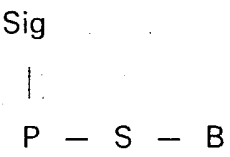


wherein P is the phosphoric acid moiety, S the sugar moiety and B the base moiety, the phosphoric acid moiety being attached at the 2', 3' and/or 5' position of the sugar moiety, said base B being attached from the N1 position or the N9 to the 1' position of the sugar moiety when said base is a pyrimidine or a purine, respectively, and wherein said Sig is a chemical moiety covalently attached to the sugar S, said Sig, when attached to said sugar S, being capable of signalling itself or making itself self-detecting or its presence known.

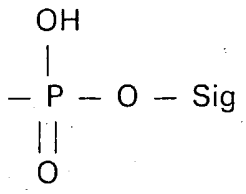
[emphasis added]

vii) Originally filed claim 141:

141. ***A nucleotide*** having the general formula



wherein P is the phosphoric acid moiety, S the sugar moiety and B the base moiety, the phosphoric acid moiety being attached to the 3' and/or the 5' position of the sugar moiety when said nucleotide is deoxyribonucleotide and at the 2', 3' and/or 5' position when said

> nucleotide is a ribonucleotide, said base B being a purine or pyrimidine, said base B moiety being attached from the N1 or the N9 position to the 1' position of the sugar moiety when said base B is a pyrimidine or a purine, respectively, and wherein ***Sig is a chemical moiety is covalently attached to the phosphoric acid moiety*** via the chemical linkage
>
> 
>
>
> said Sig, when attached to said phosphoric acid moiety P being capable of signalling itself or making itself self-detecting or its presence known. [emphasis added]

15. In the case of iv) above, the above-quoted passage plainly omits from the definition of the Sig component all practices not "in accordance with the practices of this invention." One such practice readily recognized by a person of skill in the art as not being in accordance with the invention is the alleged embodiment where the Sig component comprises a nucleotide. The passages in Paragraph 14 above make it clear that *Sig is covalently attached to the P, S or B moieties*. Apart from the inclusion of Sig, the structures given above show a nucleotide with its three subunits. If Sig were to comprise a nucleotide, then Sig would necessarily comprise the three subunits for a nucleotide. There is no description in the '069 specification, including any of the passages quoted above, that shows Sig to comprise the three subunits required for the nucleotide structure. As explained above, none of the twenty different examples of Sig that are bolded in the above-quoted passage comprise a nucleotide or the three subunits of a nucleotide.

BECAUSE THE '069 SPECIFICATION REFERS IN SEVERAL INSTANCES TO A "SIG-CONTAINING NUCLEOTIDE," OR TO "THE RESULTING NUCLEOTIDE," SIG CANNOT LOGICALLY BE NUCLEOTIDYL OR A NUCLEOTIDE ITSELF

16. Furthermore, it is clear from the passages quoted in Paragraph 14 above that

the "Sig component" of the nucleotide is not itself a nucleotide. If the Sig component comprised a nucleotide, the reference in the above quoted passages to Sig as a *component* of "the nucleotide" would make no sense. Indeed, the '069 specification is replete with evidence that the Sig component cannot comprise a nucleotide. In several instances, the '069 specification refers to "Sig-containing nucleotide" or to "the resulting nucleotide" (and the like). Some examples are provided below.

i) Page 95, lines 2-13:

> The Sig chemical moiety is covalently attached to the phosphoric acid P moiety (PM) via the [phosphate] chemical linkage . . . said Sig, when attached to said P moiety (PM) being capable of signalling itself or making itself self-detecting or its presence known and desirably ***the nucleotide*** is capable of being incorporated into a double-stranded polynucleotide . . . [emphasis added]

ii) Page 96, lines 12-20

> The chemical moiety Sig so attached to the nucleotide P-S-B (PM-SM-BASE) is capable of rendering or making the ***resulting nucleotide***, now comprising P-S-B (PM-SM-BASE) with the Sig moiety being attached to one or more of the other moieties, self-detecting or signalling itself or capable of making its presence known per se, when incorporated into a polynucleotide. . . [emphasis added]

iii) Page 96, lines 22-28

> The Sig moiety desirably should not interfere with the capability of ***the nucleotide*** to form a double-stranded polynucleotide containing the ***special Sig-containing nucleotide*** in accordance with this invention and, when so incorporated therein, the ***Sig-containing nucleotide*** is capable of detection, localization or observation. [emphasis added]

iv) Page 99, lines 6-10

> As indicated, such probes may contain one or more of the ***special Sig-containing nucleotides*** in accordance with this invention, preferably at least about ***one special nucleotide*** per 5-10 of the nucleotides in the probe. [emphasis added]

17. The just-quoted passages refer to the Sig-containing nucleotide as "the nucleotide," "the special Sig-containing nucleotide," "one special nucleotide," and

"the resulting nucleotide," If the Sig component itself were a nucleotide, these passages would make little, if any sense. There would be no "resulting nucleotide," rather, there would be a "resulting *di*nucleotide." Further, there would be no "one special nucleotide," but rather "a special *di*nucleotide." To illustrate this point more clearly, let us presume that the Sig component of "the nucleotide" were itself a nucleotide. From this presumption, it follows that every reference in the specification to "a Sig-containing nucleotide" or the like necessarily refers to a "nucleotide-containing nucleotide," or to an oligo- or polynucleotide. This leads to the anomalous result that the modified nucleotide of the invention is really not a nucleotide at all but, rather, is a *poly*nucleotide or a dinucleotide of sorts. This defies both the dictionary[13] and commonsense. Further, it contravenes the '069 specification which, as shown above, makes clear that "Sig-containing nucleotide" refers to a single nucleotide, and not a polynucleotide or a dinucleotide. The '069 specification never suggests, explicitly or implicitly, that the "Sig-containing nucleotide" could be a polynucleotide or dinucleotide. Indeed, as shown above, the '069 specification teaches that the "Sig-containing nucleotide" is "*one* special nucleotide". Accordingly, one skilled in the art would recognize that the "Sig-containing nucleotide" of the claimed invention refers to a single nucleotide and thus that Sig cannot logically be nucleotidyl or a nucleotide itself.

18. In summary, it is my opinion as a person skilled in the art to which the '069 application and invention pertains, that the recited Sig component cannot be

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[13] According to the American Heritage® Dictionary of the English Language (4th Ed. 2000), "nucleotide" is defined as "[a]ny of various compounds consisting of a nucleoside combined with a phosphate group and forming the basic constituent of DNA and RNA." The dictionary defines "oligonucleotide" as "a short polymer of *two to twenty nucleotides*." Finally, "polynucleotide" is defined as a "polymeric compound, usually DNA or RNA, consisting of a *number of nucleotides*." [emphasis added]

nucleotidyl or a nucleotide. As explained above, the various examples of Sig that are described in the '069 specification do not fit the definition of a nucleotide because the three nucleotidyl subunits necessary to constitute a nucleotide are altogether lacking in the Sig descriptions. Furthermore, as I explained above, to assert that the claimed Sig component is a nucleotide would lead to the erroneous and unreasonable conclusion that Sig can be at the same time both a component of a nucleotide (much like the sugar, phosphate and base moieties), and a nucleotide itself. A reading of the '069 specification clearly shows that Sig can only be a component of a nucleotide, and that it is not nucleotidyl or a nucleotide. Moreover, as explained earlier, Sig cannot be a nucleotide because it would force a person skilled in the art to define wrongly in several instances the word "nucleotide" in the '069 specification to mean or to refer to "a polynucleotide," "an oligonucleotide," or to "a dinucleotide."

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issued thereon.

9/27/04
Date

Dr. Alex A. Waldrop, III

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Curriculum Vitae of Alex A. Waldrop, III

ADDRESS

260 Westbrook Street #10
South Portland, Maine 04106-3350
(207) 773-6450 (207) 767-4800
(207) 767-4306 fax
awaldrop@maine.rr.com or alexw3@hotmail.com

EDUCATION

Ph.D. (Biophysics) 1977, Johns Hopkins University, Baltimore, Maryland,
Thesis Advisor: Dr. Michael Beer,
Thesis Title: "Chemical Studies "Chemical Studies of *bis*(Pyridine)osmate(VI) Esters and the Mercury Enhancement of Osmium Labelling of Polynucleotides" Dissertation Abstracts International 38 (11-B):5354+ (194 pp.) (1978);
B.S. (Chemistry) 1970, Magna cum Laude, University of Virginia.

HONORS

Echols Scholar, Phi Eta Sigma, Hugh Miller Spencer Scholarship in Chemistry, 1970.

PROFESSIONAL MEMBERSHIPS

Alpha Chi Sigma, Sigma Xi, AAAS, AACC, American Chemical Society.

EXPERIENCE

Founder and Principal Scientist, Started Company at Center for Environmental Enterprise (CEE), 2000 to present. Further characterized 9-Acridinecarbonylimidazole (AcriGlow 301) and its reaction with peroxide in various buffers and solvents. Examined ways of removing peroxide impurities from solvents, detergent and polymer solutions. Tested screening assay for detecting pollutants in environmental water samples. Served as consultant for Brims Ness, Capricorn Products, Inc., Maine Standards, and Enzo Biochem, Inc.

Visiting Scientist, Maine Medical Center Research Institute, 1994 to 2000. Synthesized and characterized modified acridancarboxylic acid ester. Demonstrated substrate activity with HRPO. Invented and characterized activated 9-acridinecarboxylic acid derivatives. Demonstrated high sensitivity assay of glucose oxidase and alkaline phosphatase. HPLC of acridine derivatives. HPLC of synthetic oligonucleotides.

Research Scientist, IDEXX Laboratories, Inc., 1992 - 1993.
Optimization of HRPO assay systems.

Staff Scientist, Gen-Probe, Inc., 1985 - 1992. Synthesized and designed acridinium esters. Helped design linker arms, optimize detection of

acridinium esters, stabilize acridinium esters, improve elution of nucleic acids from solid supports. Characterized acridinium esters by HPLC, UV and chemiluminescence.

Research Associate, Department of Microbiology, University of Virginia, 1982 - 1985. Developed new DNA sequencing method similar to Sanger approach, but which leaves functional 3' ends, which can be ligated to produce a set of deletion mutants or can be extended under conditions forcing misincorporation to generate a set of point mutations. Synthesized series of 5'-thymidine triphosphate derivatives containing a 3'-phosphate mono-, di-, or triester group. Showed that these analogs were not substrates for T4 or Klenow DNA polymerase. Developed simple, rapid gel filtration method for purifying and desalting nucleotides. Synthesized an analog of dUTP containing an EDTA group and showed that it can be enzymatically incorporated into DNA.

Assistant Professor, Department of Chemistry, University of Virginia, 1980-1982. Prepared nucleotide derivatives of tubercidin. Characterized allylamine derivatives. Taught biophysical chemistry.

Postdoctoral Research Fellow, Department of Molecular Biophysics and Biochemistry (laboratory of Dr. David C. Ward), Yale University, 1977-1980. Synthesized modified pyrimidines to incorporate in vitro into nucleic acids, using reactions between heavy metals and nucleic acid components. Developed nucleotide analogs used for gene detection in situ. Biotinyl nucleotides now selling commercially.

Predoctoral Fellow, Department of Biophysics (laboratory of Dr. Michael Beer), Johns Hopkins University, Baltimore, Maryland, 1970-1977. Developed multiple heavy atom stains for electron microscopy of nucleic acids.

ACHIEVEMENTS Co-inventor of non-radioactively-labeled nucleotides, including biotinyl nucleotides (U.S. Patents Nos. 4,711,955; 5,328,824; 5,449,767; and 5,476,928). Co-inventor of activated 9-acridinecarboxylic acid chemiluminescent system. Experienced in chemistry of nucleic acids and proteins, especially the synthetic chemistry of nucleotides, peptides, and their oligomers, and in the chemistry of mercury, osmium, and palladium; familiar with NMR, UV-Visible, IR, and fluorescent spectroscopic techniques, and with TLC, HPLC, gel filtration, and ion exchange chromatographic procedures; experienced in the use of DNA polymerases and nucleases. Experienced in detection systems for nucleic acids, especially chemiluminescence. Experienced in chemistry of acridine and acridinium compounds. Experienced with several ELISA enzymes,

including horseradish peroxidase (HRPO), alkaline phosphatase, glucose oxidase, and β–galactosidase.

Publications

(1) Richardson, F.S., Shillady, D.D., Waldrop, A.A.; A Theoretical Study of Cis-Trans Photoisomerization in the Bis(Glycinato) Platinum(II) Complex, Inorganica Chimica Acta, 5, 279-289 (1971).

(2) Waldrop, A.A., Beer, M., Marzilli, L.G.; Osmium-labeled Polynucleotides. Incorporation of Additional Heavy Atoms (Mercury) via Ligand Substitution Reactions, Journal of Inorganic Biochemistry, 10, 225-234 (1979).

(3) Langer P.R., Waldrop, A.A., and Ward, D.C.; Enzymatic Synthesis of Polynucleotides Containing Biotin: Novel Nucleic Acid Affinity Probes, Proc. Natl. Acad. Sci. U.S.A., 78, 6633-6637 (1981).

(4) Hammond, Philip W.; Wiese, Wendy A.; Waldrop, Alex A., III; Nelson, Norman C.; Arnold, Lyle J., Jr.; Nucleophilic Addition to the 9 Position Of 9-Phenylcarboxylate-10-Methylacridinium Protects Against Hydrolysis of the Ester, J. Biolumin. Chemilumin. 6(1), 35-43, (1991).

(5) Waldrop, Alex A., III; Fellers, Jonathan; Vary, Calvin P. H.; Chemilumminescent Determination of Hydrogen Peroxide with 9-Acridinecarbonylimidazole and Use in Measurement of Glucose Oxidase and Alkaline Phosphatase Activity, Luminescence 15(3), 168-182, (2000).

Patents and Patent Appplications

(1) Ward, D.C., Langer, P.R., and Waldrop, A.A.; Modified Nucleotides and Methods of Preparing and Using Same, U.S. Patent 4,711,955 (December 8, 1987). (European Pat. Appl. EP 63879 A2)

(2) Arnold, Lyle J., Waldrop, Alex A., III, Hammond, Philip W.; Protected Chemiluminescent Labels, U. S. Patent # 4,950,613 (Aug. 21, 1990). (European Pat. Appl. EP 330433 A2).

(3) Ward, D.C., Langer, P.R., and Waldrop, A.A.; Methods of Using Labeled Nucleotides. U.S. Patent #5,328,824 (July 12, 1994).

(4) Ward, D.C., Langer, P.R., and Waldrop, A.A.; Modified Polynucleotides and Methods of Preparing Same. U.S Patent #5,449,767 (Sept.12, 1995).

(5) Ward, D.C., Langer, P.R., and Waldrop, A.A.; Modified Nucleotides and Polynucleotides and Complexes Form Therefrom. U.S Patent #5,476,928 (Dec.19, 1995).

(6) Arnold, Lyle, J., Jr.; Nelson, Norman C.; Reynolds, Mark A.; Waldrop, Alex A., III; Polycationic Supports and Nucleic Acid Purification, Separation and Hybridization. U. S. Patent #5,599,667 (Feb 4, 1997). (European Pat. Appl. EP 281390 A2).

(7) Waldrop, Alex A., III and Vary, C.P.H., Peroxide-Based Chemiluminescent Assays and Chemiluminescent Compounds Used Therein. Patent pending (Submitted 1997 as Provisional Patent Application).

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Claim 1298 (CURRENTLY AMENDED). A process for detecting a nucleic acid of interest in a sample, which process comprises:

(a) providing a sample which may contain a nucleic acid of interest;

(b) specifically hybridizing said nucleic acid of interest in the sample with one or more detectable non-radioactive labeled oligo- or polynucleotides, each such oligo- or polynucleotide being complementary to or capable of hybridizing with said nucleic acid of interest or a portion thereof, wherein said oligo- or polynucleotides comprise one or more detectable non-radioactive modified or labeled nucleotides or modified or labeled nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA, and wherein said modified or labeled nucleotides or modified or labeled nucleotide analogs comprise a nucleotide structure or nucleotide analog structure which comprises one or more of :

(i) a nucleotide structure or nucleotide analog structure having the formula

PM—SM—BASE—Sig

wherein

PM is a phosphate moiety,

SM is a furanosyl moiety,

BASE is a pyrimidine, a purine or a 7-deazapurine base moiety; and

Sig is a detectable non-radioactive non-nucleotidyl moiety that comprises at least three carbon atoms,

wherein PM is covalently attached to SM, BASE is covalently attached to SM, and Sig is covalently attached to BASE directly or through a linkage group at a position other than the C5 position when BASE is a pyrimidine moiety, at a position other than the C8 position when BASE is a purine moiety and at a position other than the C7 position when BASE is a 7-deazapurine moiety, and such covalent attachment does not substantially interfere with double helix formation or nucleic acid hybridization;

(ii) a nucleotide structure or nucleotide analog structure having the formula

Sig
|
PM—SM—BASE

wherein

PM is a phosphate moiety,

SM is a furanosyl moiety,

BASE is a base moiety, and

Sig is a detectable non-radioactive non-nucleotidyl moiety that comprises at least three carbon atoms, wherein PM is covalently attached to SM, BASE is covalently attached to SM, and Sig is covalently attached to SM directly or through a linkage group and such covalent attachment does not substantially interfere with double helix formation or nucleic acid hybridization; or

(iii) a nucleotide structure or nucleotide analog structure having the formula

Sig—PM—SM—BASE

wherein

PM is a phosphate moiety,

SM is a furanosyl moiety,

BASE is a base moiety, and

Sig is a detectable non-radioactive non-nucleotidyl moiety that comprises at least three carbon atoms,

wherein PM is covalently attached to SM, BASE is covalently attached to SM, and Sig is covalently attached to PM directly or through a linkage group, and such covalent attachment does not substantially interfere with double helix formation or nucleic acid hybridization;

provided that when said nucleotide or nucleotide analog structure (iii) is attached to an oligoribonucleotide or a polyribonucleotide, and provided that when Sig is attached through a chemical linkage to a terminal PM at the 3′ position of a terminal ribonucleotide, said chemical linkage is not obtained through a 2',3' vicinal oxidation

of a 3′ terminal ribonucleotide previously attached to said oligoribonucleotide or polyribonucleotide; and

(b) detecting non-radioactively the presence of Sig in any of the detectable non-radioactive labeled oligo- or polynucleotides which have hybridized to said nucleic acid of interest.

Claim 1582 (PREVIOUSLY PRESENTED). A process for preparing a detectable non-radioactively labeled oligo- or polynucleotide of interest, comprising:

(A) providing either:

(1) one or more detectable non-radioactive chemically modified or labeled nucleotides or detectable non-radioactive chemically modified or labeled nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA or an oligo- or polynucleotide of interest alone or in conjunction with one or more other modified or unmodified nucleic acids comprising nucleotides, oligonucleotides, polynucleotides or combinations thereof, wherein said other modified or unmodified nucleic acids are capable of incorporating into an oligo- or polynucleotide of interest, and wherein said chemically modified or labeled nucleotides or modified or labeled nucleotide analogs comprise one or more signalling moieties which are capable of providing directly or indirectly a detectable non-radioactive signal; or

(2) an oligo- or polynucleotide comprising one or more said chemically modified or labeled nucleotides or modified or labeled nucleotide analogs, alone or in conjunction with one or more other modified or unmodified nucleic acids comprising nucleotides, oligonucleotides or polynucleotides;

wherein said chemically modified or labeled nucleotides or chemically modified or labeled nucleotide analogs of (1) and (2) have been modified or labeled on at least one

of the furanosyl moiety, the phosphate moiety, or the base moiety and comprise a nucleotide structure or nucleotide analog structure comprising:

(i)

PM—SM—BASE—Sig

wherein

PM is a phosphate moiety,

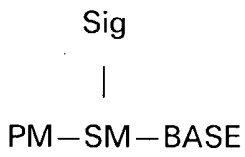
SM is a furanosyl moiety,

BASE is a pyrimidine, a purine or a 7-deazapurine base moiety, and

Sig is a detectable non-radioactive non-nucleotidyl moiety that comprises at least three carbon atoms, and

wherein PM is covalently attached to SM, BASE is covalently attached to SM, and Sig is covalently attached to BASE directly or through a linkage group at a position other than the C5 position when BASE is a pyrimidine moiety, at a position other than the C8 position when BASE is a purine moiety, and at a position other than the C7 position when BASE is a 7-deazapurine moiety;

(ii)



wherein

PM is a phosphate moiety,

SM is a furanosyl moiety,

BASE is a pyrimidine, a purine or a 7-deazapurine base moiety, and

Sig is a detectable non-radioactive non-nucleotidyl moiety that comprises at least three carbon atoms , and

wherein PM is covalently attached to SM, BASE is covalently attached to SM, and Sig is covalently attached to SM directly or through a linkage group; or

(iii)

Sig—PM—SM—BASE

wherein

PM is a phosphate moiety,

SM is a furanosyl moiety,

BASE is a pyrimidine, a purine or a 7-deazapurine base moiety, and

Sig is a detectable non-radioactive non-nucleotidyl signalling moiety that comprises at least three carbon atoms and is detected non-radioactively by an enzymatic measurement, a fluorescent measurement, a chemiluminescent measurement, an electron density measurement, a magnetic measurement, or any combination of the foregoing measurements; and

wherein PM is covalently attached to SM, BASE is covalently attached to SM, and Sig is covalently attached to PM directly or through a linkage group;

provided that when said nucleotide structure or nucleotide analog structure (iii) is attached to an oligoribonucleotide or a polyribonucleotide, and provided that when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not obtained through a 2',3' vicinal oxidation of a 3' terminal ribonucleotide previously attached to said oligoribonucleotide or polyribonucleotide; and

said oligo- or polynucleotide of interest; and

(B) either incorporating said chemically modified or labeled nucleotides or nucleotide analogs (A)(1) into said oligo- or polynucleotide, and preparing a non-radioactive labeled oligo- or polynucleotide of interest, or incorporating or attaching chemically modified or labeled nucleotides or nucleotide analogs or unmodified nucleotides or nucleotide analgs to said oligo- or polynucleotide provided in said incorporate or attach step (A)(2) above, thereby preparing a non-radioactive labeled oligo- or polynucleotide of interest.

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